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Ameliorative Effect of Cannabidiol against Gastric Ulcer Induced by Diclofenac in Rats

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Purpose: The aim of his study is to examine the effect of using cannabidiol upon gastric ulcer induced by diclofenac in rats. Method: The experiment was conducted on four groups, with normal rats serving as a control in group one. A single oral dose of diclofenac was administered to Group 2 rats in order to induce a stomach ulcer. The rats in the third group were given a single oral dosage of diclofenac sodium, followed by a 5-day therapy with cannabidiol, which began three days before the diclofenac administration. Drug control on cannabidiol-treated rats in the final group (Group 4). Results: The investigation showed that when cannabidiol was administered, the levels of acidity from diclofenac use decreased, significantly. Conclusion: The investigation resulted in a decrease in overall acidity levels among the rats treated with diclofenac, indicating that cannabidiol was effective in treating the condition.

Keywords: Gastric ulcer; gastric acidity; toxicity; diclofenac and cannabidiol.

1. INTRODUCTION

Diclofenac is a nonsteroidal, anti-inflammatory medication that act by reducing the pain or inflammation causing substances from the body. It can be used to treat mild to moderate pain or some signs of rheumatoid arthritis or osteoarthritis. Before use of the drug it is important to note if one has any allergic reaction to non-steroidal anti-inflammatory drugs. Diclofenac is maily used for pain relief escpecially in case of artritis. A decline in these

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symptoms aids in the improvement of normal body activities [1]. The medical provision for this case is termed as a nonsteroidal anti-inflammatory drug (NSAID). The treatment of arthritis requires an understanding of the need for diclofenac medications in individuals for pain relief and treatment of other pre-existing medical conditions [1]. A medication guide issued by medics, with more emphasis offered by the existing pharmacists, guides the use of Diclofenac Sodium. The medication is administered by mouth, with a glass of water of 8 ounces, unless the doctors in charge make changes [2]. After taking the drug, an individual is expected not to lie down for at least 10 minutes. Problems taking the drug call for complementing with food, milk or any form of anti-acid. Nevertheless, this approach delays relief from pain since the absorption is quite slow. Diclofenac exists in different strength. First, Diclofenac Sodium 25Mg Tablet, Delayed Release, round in shape and yellow in colour with an imprint of GG 737. Secondly, Diclofenac Sodium 75Mg Tablet, Delayed Release, round in shape and light pink with an imprint of GG 739 [2]. Thirdly, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and light brown with an imprint of GG 738. Fourthly, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and white with an imprint of WPI 338. Fifth, Diclofenac Sodium 25Mg Tablet, Delayed Release, round in shape and light brown with an imprint P 25 [3]. Sixth, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and light with an imprint of P 50. Seventh, Diclofenac Sodium 75Mg Tablet, Delayed Release, round in shape and light brown with an imprint of P 75 [4]. Eighth, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and brown with an imprint of GG 738. Ninth, Diclofenac Sodium 75Mg Tablet, Delayed Release, round in shape and light pink with an imprint of G-DS-75. Tenth, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and light brown with an imprint of G-DS-75. Eleventh, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and light brown with an imprint of G-DS-50 [4]. Twelfth, Diclofenac Sodium 75Mg Tablet, Delayed Release, round in shape and white with an imprint of 551 logos. Thirteenth, Diclofenac Sodium 50Mg Tablet, Delayed Release, round in shape and white with an imprint of 551 logos.

On the side effects, Diclofenac results in stomach upsets, heartburn, nausea, diarrhoea, constipation, gas, drowsiness, dizziness, headache, cramping, abdominal burning, gastrointestinal bleeding, and liver toxicity [5]. At times the stomach ulceration may occur without any abnormal pain. There could also be rash, kidney impairment, lightheadedness and ringing ear. Furthermore, it leads to high blood pressure among the respective users [6]. It also results in hearing challenges, mood changes, painful swallowing, heart failure such as unusual fatigue is also associated with the medication on the users.

Marijuana has been found as the main source of Cannabinoids. Marijuana is also known as hashish and flowers of Cannabis, which are extracted from the Cannabis sativa plant in the form of cannabis resin [7]. The plant comprises over 80 phytocannabinoids. The major component of marijuana comprises psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC), which acts as cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2) receptors can be considered as a partial agonist [8]. Some of the other cannabinoids within marijuana comprise non-psychoactive cannabidiol (CBD), Δ^9-tetrahydro-cannabivarin (Δ^9-THCV) and cannabichromene (CBC) [9]. Of all the existing components, CBD has been of the highest attention, with the realization of the antagonist effect of CB_1/CB_2 receptor agonists in countering psychotropic, as well as negative implications of Δ^9-THC [10]. The existing data show that there exists an inverse behaviour agonist of CB_1 and CB_2 receptors. The cannabinoids obtained from plants are used in medicinal run-through, for instance, Δ^9-THC (dronabinol) and its artificial replica, nabilonein contradiction of chemotherapy-induced biliousness and emesis, and to stimulate appetite such on patients suffering from AIDS. CBD in combination with Δ^9-THC (nabiximols) aid in neuropathic pain relief, based on a series of sclerosis used as analgesia for the treatment of severe cancer pain. Furthermore, phytocannabinoids, which is a component of organic materials, remains in the close alignment of cannabinoid receptors, which can be considered as endocannabinoids. The phospholipid mediators are not stored, yet synthesize based on demand within a site and are dependent on time, increasing greatly through a series of degradation based on transient and localized impacts [11]. The discovery of Cannabinoid receptors' took place, after the discovery of the endogenous ligands' isolation, which is termed as the endocannabinoids such as the CB_1 receptor. CB_2 receptor underwent cloning back in 1993, as the second endocannabinoid, 2-arachidonoylglycerol

(2-AG) acknowledged in Pertwee [12]. After that period, more endogenous cannabinoids have been realized such as homo-γ-linolenoylethanolamine, 7,10,13,16-docosatetraenoylethanolamide,2-rachidonoylglycerol ether (2-AGE, noladin ether), O-arachidonoyl ethanolamine (virodhamine) and N-arachidonoyl dopamine (NADA). Moreover, endogenous cannabinoids and synthetic derivatives act as receptors such as AEA with methanandamide to improve intake of inhibitor AM404 (N-arachidonoylaminophenol) in activating TRPV1 receptor, and AEA [13]. The cannabinoids at as putative for non-CB_1, non-CB_2, non-TRPV1 receptors, putative non-I_1, non-I_2 imidazoline receptors and putative allosteric sites for muscarinic M_1 and M_4 receptors and 5-HT_2, 5-HT_3 receptors.

The main purpose of the experiment was to identify the effect of cannabidiol on rats and then investigate the effect of cannabidiol upon the gastric toxicity induced by diclofenac where it synergistic or inhibition impact.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Cannabidiol powder and diclofenac from Cayman Chemical Establishment (USA) was primed in 1% aqueous solution, Tween 80. Diclofenac (sigma).

2.2 Animals

The study used four groups of rats (n=6 rats per every group). The rats were randomly assigned the groups to be given different treatments for the specified study timelines.

On the animals, masculine Sprague-Dawley rats weighing 250 ± 10 g. Animal House in the College of Medicine, King Faisal University ensured all the animals for use are properly availed [10]. The typical lodging amenities (24 ± 1◦C) was used for all the animals. A supply of 45 ± 5% moistness and 12 h well-lit/dusky cycle was provided with the ordinary laboratory nosh and water ad libitum left to enhance acclimatization for a week before the experiment.

The Ethical Committee, Deanship of Scientific Research, King Faisal University, approved permission for the experiment. The procedure on experiments was undertaken in line with international standards for care and laboratory animals. The experiments were done in 4 groups; groups 1, 2,3 and 4.

2.3 Experimental Design

The study adopted a true experimental controlled research design. This is because treatments were provided to different groups of rats at different levels and one group was not treated to be used as a control to compare the differences in acidity and mean ulcer index values. In group 1, normal rats have used a control. Group 2 rats were induced for gastric ulcer with a single oral dose of diclofenac sodium 80 mg/kg body weight in water) for 48 hours fasting for DIC. In the third group, the rats were induced for gastric ulcer using diclofenac sodium's single dose which contains 80 mg/kg body in water after 48-hour fasting and treatment are done using cannabidiol of 5 mg kg-1/day applied for 5 days, commencing with 3 days before diclofenac administration. In the last group (Group 4), drug control on the rats was cured using cannabidiol (5 mg kg-1/day, i.p was offered for 5 days (Fig. 1).

2.4 Collection of Gastric Juice

The stomach of each of the rats was immediately excised, making the oesophagus closed, with all the gastric contents collected and underwent centrifugal at 3000 rpm for 10 minutes for the removal of any traces of solid debris and the volumes of the supernatant measured.

2.5 Measurement of Gastric Acidity

Later, the volumes of the supernatant were measures and expressed as mL/100g with the pH examination done. After that, curvature, which has been washed with saline ice-cold water, was used for opening the stomachs of the object. This was followed by an examination of macroscopical mucosal lesions.

Ulcer index (UI) was calculated using some scoring system. The ulcerative lesions underwent classifications such as;

$$Normal\ stomach = 0; spot\ ulceration = 1.0; haemorrhagic\ streaks = 1.5; ulcers = 2.0$$

Percentage inhibition was later calculated from the realized results.

$$\%\ Inhibition\ of\ alceration = (UICONTROL - UI\ TEST) \div UI\ CONTROL x100$$

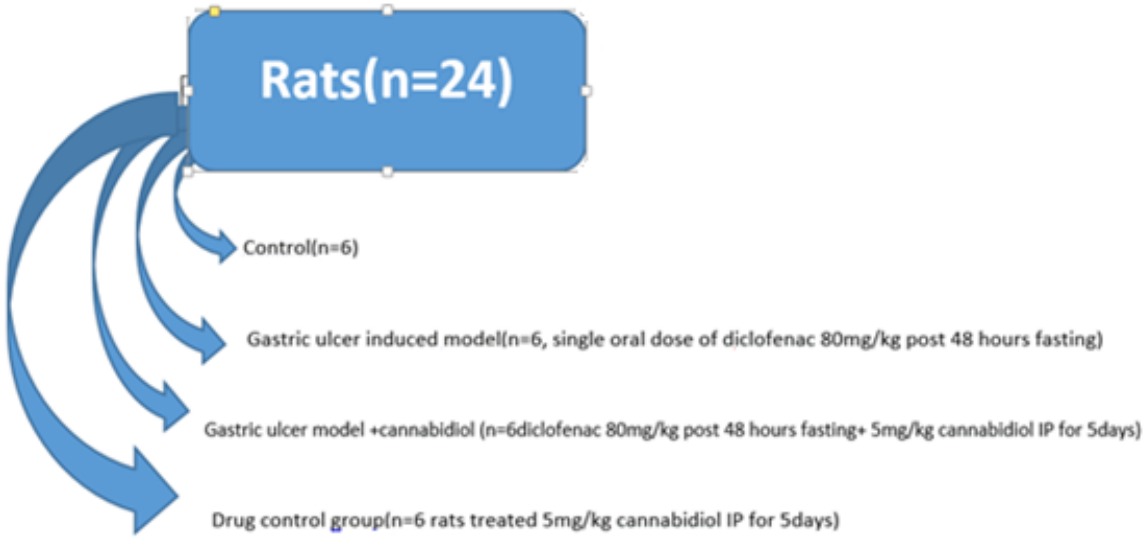

Fig. 1. Flowchat illustrating the testing process

2.6 Determination of Total Acidity

A solution of 1 mL gastric extract diluted with 1 mL of distilled water was taken into a 50 mL conical flask, followed by the addition of two droplets of phenolphthalein indicator (Devane et.al, 1988). The flask was titrated with 0.01 M NaOH until a perpetual pink colour was observed. The volume of 0.01 M NaOH was recorded. The total acidity was expressed as mEq/L by the following formula;

$$Acidity = Volume\ of\ NAOH\ x\ Normality\ x \frac{100}{0.1}$$

2.7 Determination of Free Acidity

Topfer's reagent was considered for the trial in place of a phenolphthalein indicator. An aliquot of gastric juices was titrated with 0.01 M NaOH until the yellow colour of the canary was observed [13]. The volume of 0.01 M NaOH consumed was recorded. For calculating the free level of acidity, a similar formula was used as it was used in determining the total acidity of the object.

2.8 Statistical Analysis

The findings from the analysis were expressed based on the average and the deviation from the mean (mean±S.E). A paired sample t-test was adopted for analysis to test for differences across the groups. The differences were considered for $p<0.05$. The calculation was based on comparing between the treatment and the control groups for the mean ulcer index and percentage of ulcer exhibition.

3. RESULTS AND DISCUSSION

Table 1. Outcome of pretreatment with omeprazole, cannabidiol free acidity, total acidity, ulcer index and per cent of ulcer inhibition in rats with diclofenac induced ulcers

Group	Treatment	Dose	Free acidity (mEq/L)	Total acidity (mEq/L)	Mean ulcer index	% of ulcer inhibition	t-stat	P
I	Control		101.3± 2.43	108.76±2.09	0	0	0.785	p>0.05
II	Diclofenac	80 mg/kg	276± 4.88	288± 1.98	9.08±0.08	0	3.566***	p<0.05
III	Standard drug (omeprazole)	80 mg/kg	36.87± 2.87	46.96±2.65	2.09±0.05	77%	5.674***	p<0.05
IV	Cannabidiol	5 mg/kg	98.67± 4.35	109.76±2.32	3.63±0.03	60%	6.764***	p<0.05

Values are expressed as mean ± SEM (n = 7). P values < 0.05 were considered statistically significant

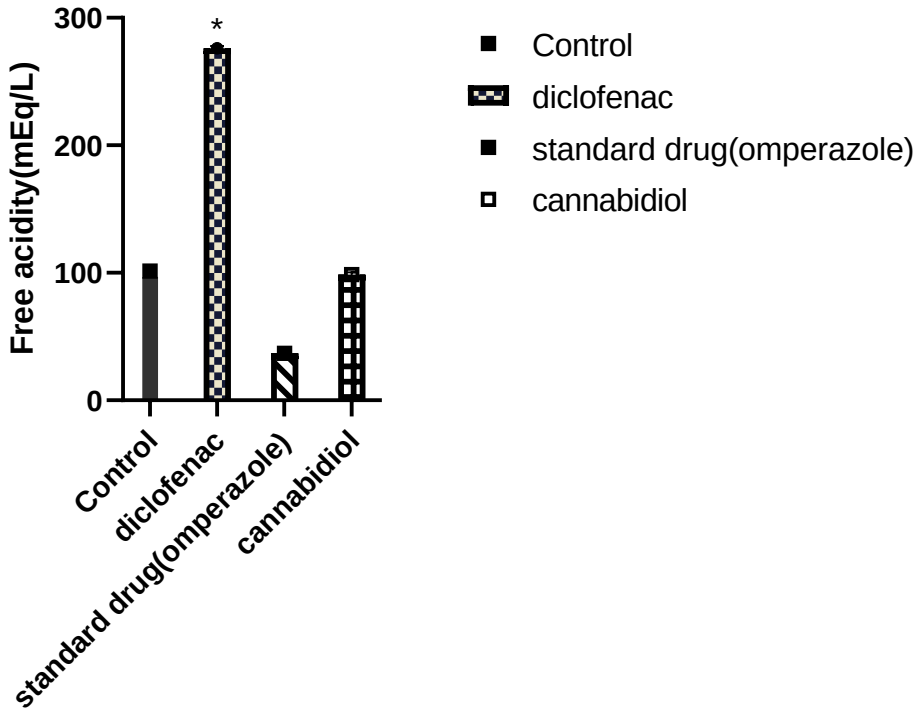

Fig. 2. Outcome of pretreatment with omeprazole, cannabidiol free acidity in rats with diclofenac induced ulcers, P values < 0.05 were considered statistically significant

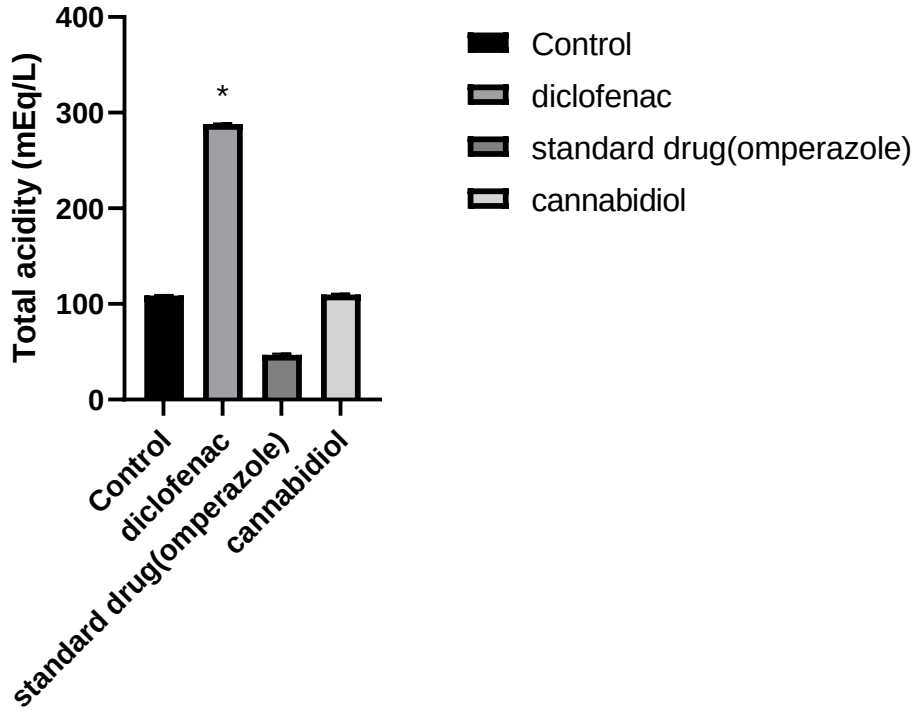

Fig. 3. Outcome of pretreatment with omeprazole total acidity in rats with diclofenac induced ulcers, P values < 0.05 were considered statistically significant

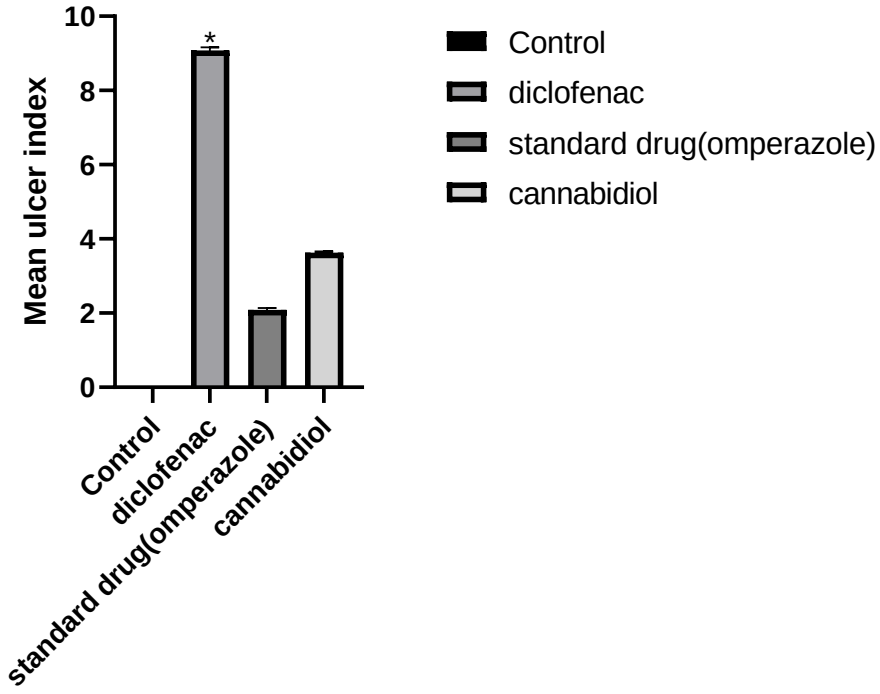

Fig. 4. Outcome of pretreatment with omeprazole mean ulcer index in rats with diclofenac induced ulcers, P values < 0.05 were considered statistically significant

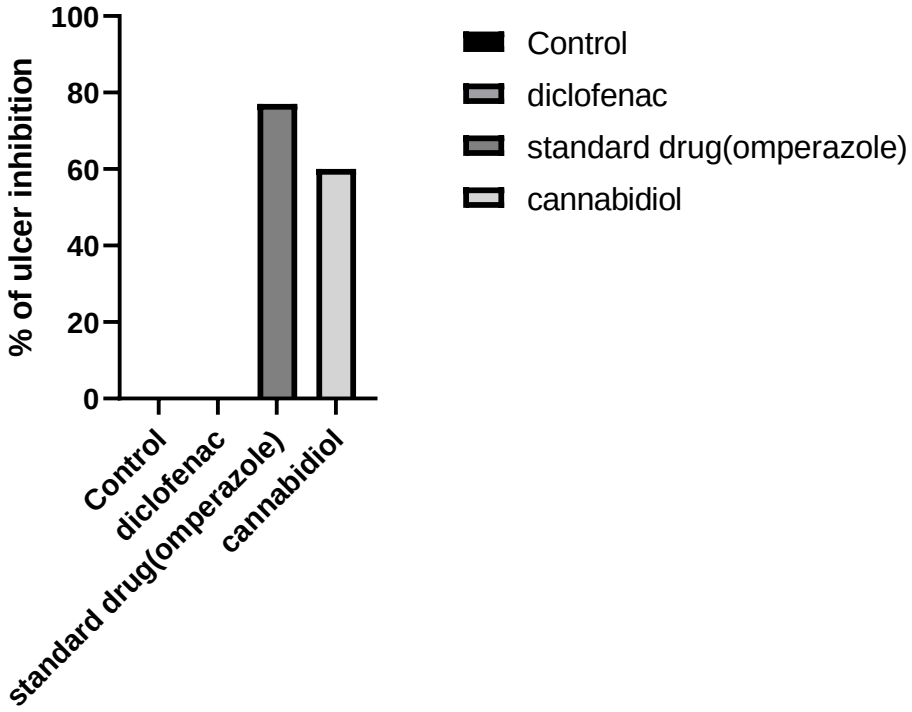

Fig. 5. Outcome of pretreatment with omeprazole mean ulcer index in rats with diclofenac induced ulcers, P values < 0.05 were considered statistically significant

On the control, no dosage was offered, resulting in a free acidity of 101.3±2.43 and total acidity of 108.76±2.09 (Figs. 2 and 3). In the second group, diclofenac was used at 80mg/kg. This resulted in a free acidity of 276±4.88, total acidity of 288±1.88 and a mean ulcer index of 9.08±0.08 (Figs. 2, 3 and 4). In the third group, the introduction of a standard drug (omeprazole) at 80mg/kg resulted in free acidity of 36.87±2.87, total acidity of 46.96±2.65, mean ulcer index 2.09±0.05 and percentage of ulcer inhibition of 77% (Figs. 2,3,4 and 5). In the last group, the introduction of cannabidiol at 5mg/kg resulted to free acidity of 98.67±4.35, a total acidity of 109.76±2.32, mean ulcer index of 3.63±0.03 and a percentage of ulcer inhibition of 60% from all the elements present in the experiment (Figs. 4 and 5). It been shown in this study when rats were given Diclofenac, a conventional medication (omeprazole), or cannabidiol, the mean ulcer index decreased (Fig. 4). Furthermore, the proportion of ulcer inhibition was observed to be the same throughout the several groups of rats used in the investigation. The experiment implicates significant variation between the control, groups 1, 2,3 and 4 respectively ($p<0.05$). The analysis noted that there was no variation in the mean ulcer and percentage of ulcer inhibition across the experimental groups ($t(5)=0.785$, $p>0.05$). Nonetheless, there existed significant differences across Diclofenac ($t(5)=3.566$, $p<0.05$), standard drug (omeprazole) ($t(5)=5.674$, $p<0.05$) and cannabidiol ($t(5)=6.764$, $p<0.05$). This suggests that diclofenac causes increased stomach acidity in rats, which is reduced by cannabidiol use. As evidenced by the findings, the majority of these changes imply possible difference in the levels of ulcers among the rats participated in the study. The presence of a significant variation in the mean ulcer and percentage of ulcer inhibition throughout the treatments for the various groups of rats suggested that the mean ulcer and percentage of ulcer inhibition may alter over time.

In this study pretreatment rats with cannabidiol has decreasing effect on free acidity, total acidity, ulcer index in rats with diclofenac induced ulcers. Intake of cannabidiol reduces the number of ulcers on the rats, hence a possible curative measure on the stomach acidic from the specific rats involved in the experience [14]. This conforms to the existing studies concerning the effect of cannabidiol on ulcers and the side effects of diclofenac on the users [15]. This is based on the capability of cannabidiol to help in reducing acidity levels in the stomach of all the rats.

Previous studies [16-21] demonstrated ulceration from both macroscopic and microscopic examinationsas results of diclofenac treatment. In study by Khan and colleagues shown using of plant called Dalbergia sissoo Roxb has ability in reducing the ulcer size and gastroproective effect ith diclofenac treatment [21]. The present study shown that treated rats with cannabidiol show promise in decreasing stomach acidity and thereby gastric ulcers with diclofenac induced gastric ulcer in rats. As seen by the provided statistics on rats, it demonstrates a significant reduction in the levels of ulcers.

4. CONCLUSION

According to the results of the study, diclofenac increases the number of gastric ulcers in the rats' stomachs. The use of cannabidiol to treat the ailment was successful, with the experiment resulting in a decrease in total acidity levels. The inclusion of a standard medicine (omeprazole) as well as cannabidiol was crucial in the reduction of the mean ulcers index in the rats. The findings showed that these medications can help protect the GI tract from bleeding and ulcers caused by NSAIDs. Because they showed substantial differences with the mean ulcer index (2.09±0.05, 3.63±0.03) for omeprazole and cannabidiol respectively, the inclusion of standard medicine (omeprazole) and cannabidiol ingredients offers a greater chance of improving the health condition of the persons against any type of ulcer. The combination of the normal medicine (omeprazole) and cannabidiol was found to be particularly effective in minimizing gastrointestinal damage caused by diclofenac thereby aiding in the protection of the ulcer-induced from Diclofenac's effects.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

In-vivo experimental approaches were approved by the institutional animal ethics committee (IAEC) from King Faisal University.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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